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Rock Island Arsenal Laboratory

TECHNICAL REPORT

ENZYMATIC DEOXYGENATION
A NEW CONCEPT IN CORROSION PREVENTION
(REPORT #2)

296 824

By

W. F. Garland

Department of the Army Project No. 593-32-007

Ordnance Management Structure Code No. 5010.11.842

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Approved by:

A. C. Hanson

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Laboratory Director

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Rock Island Arsenal
Rock Island, Illinois

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ABSTRACT

In an effort to improve the prototype deoxygenating packet, an investigation was initiated into increasing the rate of reaction and shelf life.

The feasibility of using an enzymatic deoxygenating system for providing corrosion protection was described in Rock Island Arsenal Report No. 61-3681. This process is based on the specificity of the enzyme glucose oxidase for causing molecular oxygen to be utilized in reactions, i.e.,

$$\underset{\text{glucose}}{2\ C_6H_{12}O_6} + 2\ O_2 + 2\ H_2O \xrightarrow[\text{oxidase}]{\text{glucose}} \underset{\substack{\text{gluconic}\\\text{acid}}}{2\ C_6H_{12}O_7} + 2\ H_2O_2$$

$$2\ H_2O_2 \xrightarrow{\text{catalase}} 2\ H_2O + O_2$$

Net reaction:

$$2\ C_6H_{12}O_6 + O_2 \xrightarrow[\text{system}]{\text{enzyme}} 2\ C_6H_{12}O_7$$

Three surfactants were evaluated in a saturated buffered glucose solution with a view toward increasing its absorption on the molecular sieves, and the capacity and rate of oxygen absorption of the deoxygenating medium. Surfactant A proved to be the most effective wetting agent used; increasing both the capacity and rate of the original mixture.

Four forms of molecular sieves (1/16", 1/8", powdered and "improved") a second synthetic zeolite and activated alumina were investigated as substrate material for the deoxygenating liquid. Of the several materials tested, activated alumina provided the best balance of substrate properties, inert, highly absorbant, and inexpensive. An improved deoxygenating packet utilizing activated alumina as the substrate material for the deoxygenating liquid is described.

RECOMMENDATIONS

It is recommended that further work be directed toward evaluation of the improved deoxygenating packets in sealed containers under simulated and actual packaging and storage conditions.

It is also recommended that the shelf life of up to one year of the improved packets be investigated.

Development work on the microencapsulation of the enzymatic deoxygenating solution is recommended toward production of a simple, convenient to use, all purpose, flexible packaging material.

ENZYMATIC DEOXYGENATION
A NEW CONCEPT IN CORROSION PREVENTION
(REPORT #2)

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ENZYMATIC DEOXYGENATION
A NEW CONCEPT IN CORROSION PREVENTION
(REPORT #2)

OBJECT

To develop an improved deoxygenating packet that reflects the maximum efficiency and capacity of the enzyme system.

INTRODUCTION

In general, moisture and oxygen must be present for corrosion (metallic oxidation) to occur[1,2]. In the past, the primary emphasis in the field of corrosion prevention has been placed upon keeping moisture away from the metallic item. Such efforts have resulted in numerous rust preventive oils, greases and coating compound, vapor phase inhibitors, desiccants and dehumidification techniques.

The use of an inert atmosphere for corrosion prevention has been used to a limited extent. Generally, such an atmosphere is produced mechanically by alternately evacuating and flushing a container with nitrogen. Unfortunately, quantitative oxygen removal is difficult, if not impossible, to obtain by such mechanical means and the resulting protection is not reliable. While inert gas and vacuum packaging have been extensively used in the food industry, complete removal of oxygen cannot be obtained. Very little entrapped oxygen is removed by either process.

The commercial introduction in 1952 of the enzyme glucose oxidase gave rise to a new approach to the problem of corrosion prevention.

Enzymes are proteinaceous, catalytic agents whose purpose is to accelerate the reactions that occur under the conditions existing in living matter, at a temperature compatible with life, in the presence of water and usually at a nearly neutral pH[3]. They are made only by living cells, and all living cells contain them, but they often can be extracted from their original locations and made to catalyze chemical reactions[4]. The enzyme as a catalyst only causes a reaction to occur and does not participate in the reaction and, thus, is not used up during the reaction.

As marketed, the commercial enzyme contains appreciable amounts of catalase, but only traces of other enzymes. The catalase present is advantageous for many of the industrial applications. This enzyme system catalyzes the reaction,

between glucose and oxygen with gluconic acid and hydrogen peroxide as the reaction products. Enzyme action is highly specific in nature since the cell manufactures the enzyme to accelerate a single reaction or group of very similar reactions[5]. Dependent on the conditions employed, glucose oxidase can be used to remove either glucose or oxygen from a system[6,7]. Glucose oxidase is the only known easily obtained enzyme capable of causing molecular oxygen to be utilized in a reaction. However, an antibacterial agent in culture liquor from P. notatum, called notatin[8] or penicillin B[9], has been found to be the same oxidative enzyme.

The reaction catalyzed by glucose oxidase produces hydrogen peroxide which is decomposed (as soon as it forms[10]) into oxygen and water by means of the included enzyme, catalase. The following equations illustrate the enzymatic deoxygenation reaction:

(1) $2\ C_6H_{12}O_6 + 2\ H_2O + 2\ O_2 \xrightarrow[\text{oxidase}]{\text{glucose}} 2\ C_6H_{12}O_7 + 2\ H_2O_2$

glucose — gluconic acid

(2) $2\ H_2O_2 \xrightarrow{\text{catalase}} 2\ H_2O + O_2$

Net reaction:

(3) $2\ C_6H_{12}O_6 + O_2 \xrightarrow[\text{enzyme system}]{\text{glucose oxidase-catalase}} 2\ C_6H_{12}O_7$

It will be observed that upon adding equation (1) with equation (2), half of the oxygen involved has been consumed and all of the catalyzer remains. The reaction is therefore repeated continuously, the free oxygen present being reduced one-half each time, thereby the free oxygen present rapidly approaches zero.

Glucose oxidase is used in many products in the food industry[11]. Enzymatic removal of glucose from eggs prior to drying is an important use of this enzyme. This enzyme is also used to remove oxygen from packaged foods to prevent oxidative deterioration[12].

The ability of glucose oxidase to take up oxygen was first noted in 1904 in ground mold, mycelium[13]. As used today, the enzyme is applied as a system consisting of glucose oxidase and catalase. The enzyme system is derived from a mold grown under conditions very similar to the procedures used for the production of antibiotics.

Currently, a glucose oxidase-catalase enzyme system is being used on an experimental scale in the preservation of foods and beverages with promising results[14,15,16]. Oxygen removal by this technique is completed after the package is sealed and will proceed whenever oxygen is present.

Preventing corrosion by surrounding a metallic item with an oxygen-free inert atmosphere by in-package oxygen removal offers many advantages over conventional means of protection. Metallic items need not be coated with rust preventive compounds, the technique could be used with all metals and plastics and would be noncontaminating and the items would not be subject to fungal deterioration.

Burke[17], in earlier work on enzymatic deoxygenation at Rock Island Arsenal, demonstrated that inert atmospheres produced mechanically or by effective in-package oxygen removal prevented corrosion in the presence of moisture. Further work[18] covered the evaluation of three commercially prepared deoxygenating systems and a Rock Island Arsenal Prototype deoxygenating packet. Two of the commercially prepared packets, as well as the Rock Island Arsenal prototype packet, were based on the specificity of the enzyme glucose oxidase for causing molecular oxygen to be utilized in reactions.

This report covers the work at Rock Island Arsenal in the adaptation of the enzymatic deoxygenating process to corrosion prevention.

PROCEDURE AND RESULTS

The three commercial deoxygenating systems investigated were as follows:

T-1 packet - A two-celled plastic design, one side of which consists of 0.0005 inch low density polyethylene to allow high oxygen permeability with low moisture permeability. The other side of the packet is conventional density polyethylene. One cell contains the dry ingredients; glucose, buffer salts, and absorbent media. The other cell contains the liquid enzyme preparation. Packets are activated by breaking the seal between the cells, thus allowing the components to mix.

F-1 packet - Consists of a bag of 0.00025 inch polyethylene coated porous (tea bag) paper containing the active preparation on an absorbent.

C-1 pellets - Consist of finely ground sodium sulfite and copper sulfate pentahydrate in a 2:1 ratio.

The three commercial deoxygenating systems were evaluated in nine, 9-ounce, wide mouth, screw capped jars assembled as shown in Figure 1. The glass tubing was adhered to the bottom of the jar with a plastic adhesive. The small glass vial was attached to the jar by means of a pressure sensitive tape hinge to control the direction of its fall when tipped to release the water. Disks of 1018 steel, ground, and cleaned (in boiling naphtha and methanol) were glued to the top of the glass tubing.

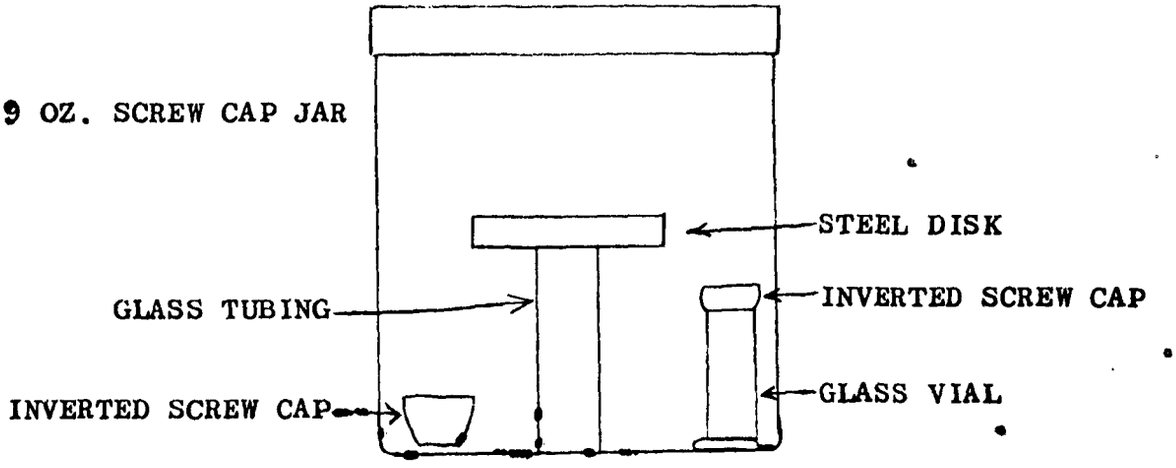


FIGURE 1

APPARATUS USED FOR TESTING IN-PACKAGING OXYGEN REMOVAL

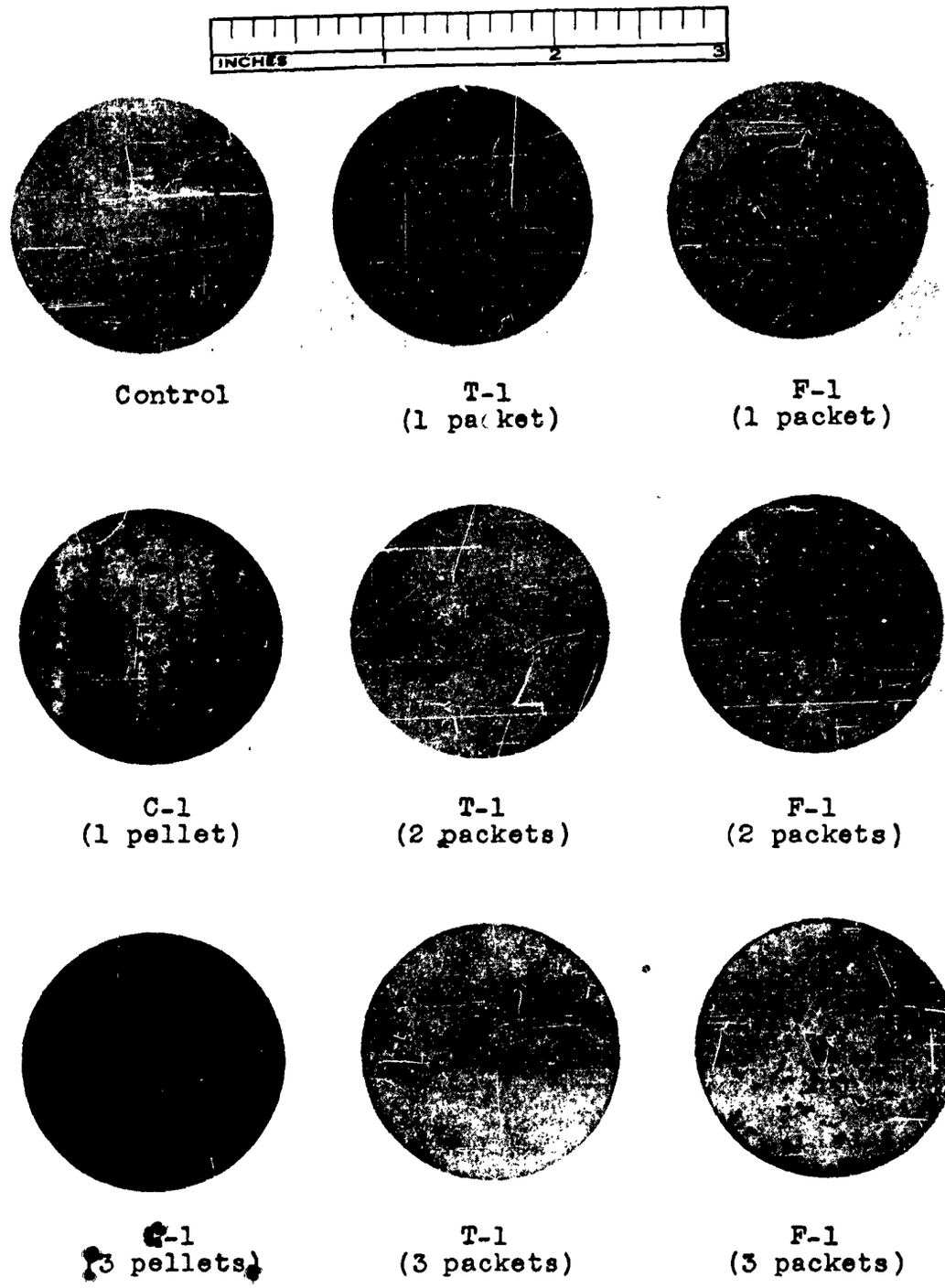
The following items were placed in each jar:

Jar No.	Items
1	No deoxygenating material, control
2	One C-1 pellet, placed in an inverted screw cap
3	Three C-3 pellets, placed in an inverted screw cap
4	One F-1 packet, taped to container wall
5	Two F-1 packets, taped to container wall
6	Three F-1 packets, taped to container wall
7	One T-1 packet, taped to container wall
8	Two T-1 packets, taped to container wall
9	Three T-1 packets, taped to container wall

Each small vial was filled with distilled water and covered with an inverted screw cap. The container lid was tightened and sealed with adhesive. After 24 hours, the container was tilted to tip over the small vial and release the water. After 64 hours at ambient temperature the disks were removed and examined.

Figure 2 shows the effects of in-package oxygen removal utilizing three commercially prepared products. Both types of packets and pellets were also tested in a manometric apparatus to determine their effectiveness of oxygen removal as will be reported elsewhere in this report. The pellets were found to be inactive and these results were confirmed in independent tests made by the manufacturer.

The T-1 and F-1 packets showed approximately the same efficiency and capacity for oxygen uptake. The difference in performance probably can be attributed either to the higher moisture permeability of the F-1 packet material or the fact that this product could emit carbon dioxide in a neutralization side reaction. High moisture permeability would allow moisture from the packet to reach the disk prior to the time deoxygenation was completed, causing corrosion. Carbon dioxide in the presence of moisture would form carbonic acid which could also attack the metal.



CORROSION EFFECTS ON STEEL DISKS AFTER DEOXYGENATION

24 hours allowed for deoxygenation; 64 hours in humidity saturated atmosphere at ambient temperature.

FIGURE 2

Neg.No. 1181
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A comparison of the efficiency of the T-1 and F-1 packets was made by placing both types of packets in 9 ounce French square bottles fitted with manometers and sealing. Open end manometers were made from 3/16" O.D., 1/8" I.D. glass tubing as shown schematically in Figure 3. Six bottles were assembled as follows: 3 bottles each containing one T-1 packet, fitted with a manometer and sealed with liquid plastic (vinyl chloride-vinyl acetate copolymer); 3 bottles, each containing three T-1 packets, fitted with manometers and sealed. The above experiment was repeated utilizing the F-1 packet material. Manometer readings were made at recorded intervals.

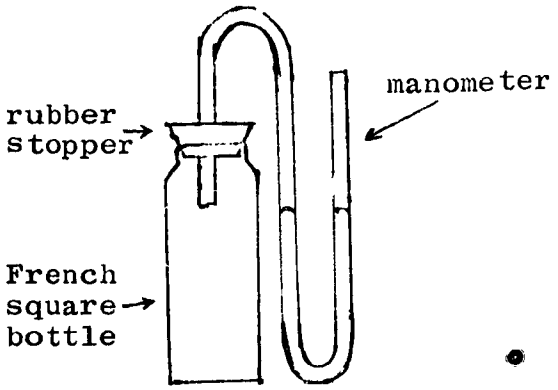


FIGURE 3

Figure 4 illustrates the extent of oxygen removal with time when using single as well as 3 packets of each type of deoxygenating material. The C-1 pellets, due to their poor performance in the corrosion test (see Figure 2), were omitted from further testing. As had been previously stated, the oxygen uptake of both types of packets was approximately the same, however, when three packets of each type were compared with each other, the T-1 packets showed a much higher efficiency and capacity after ten hours of deoxygenating. While neither type of packet reached the theoretically complete oxygen removal differential pressure (6.2 in. Hg.), the difference in capacity is readily apparent.

While the results of the previous experiment appeared promising, a great deal of difficulty was experienced in the use of both the T-1 and F-1 packets. In the former, the liquid had frequently crystallized, (and in a few cases, molded). The packet had to be activated by puncturing rather than breaking the seal. The F-1 packets had to be used immediately after removal from the shipping container to insure their activity, thus also making their use somewhat inconvenient.

It was deemed desirable then to fabricate a deoxygenating packet in an attempt to overcome the above mentioned drawbacks, thus producing an improvement over the commercial product.

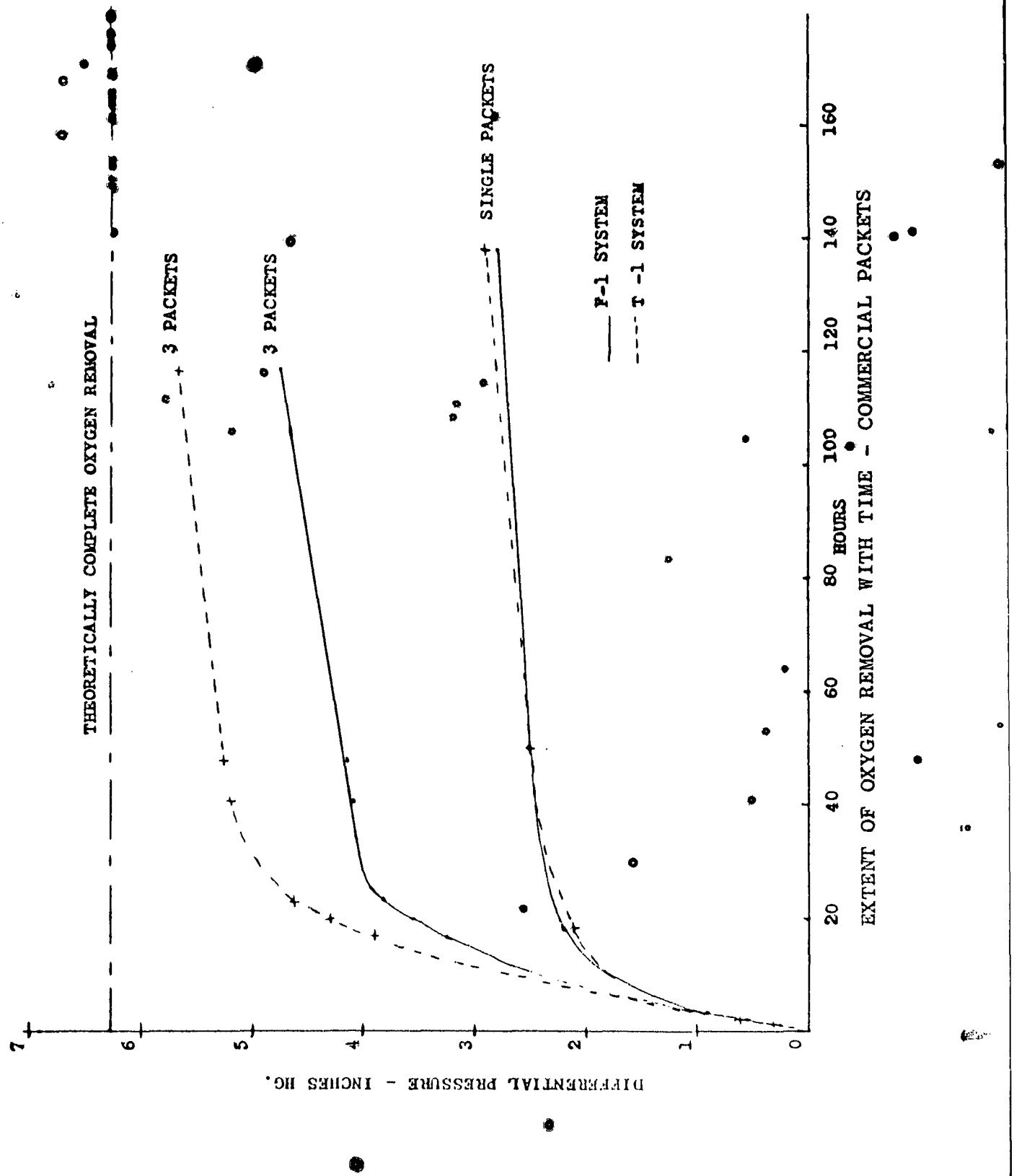

EXTENT OF OXYGEN REMOVAL WITH TIME - COMMERCIAL PACKETS
DIFFERENTIAL PRESSURE - INCHES HG.
HOURS
0
20
40
60
80
100
120
140
160
1
2
3
4
5
6
7
THEORETICALLY COMPLETE OXYGEN REMOVAL
3 PACKETS
3 PACKETS
SINGLE PACKETS
P-1 SYSTEM
T -1 SYSTEM

FIGURE 4

Prototype Packet

A liquid concentrate of the enzyme (glucose oxidase-catalase, 750 units*/milliliter) obtained from the manufacturer of the T-1 packets was utilized in designing experiments to determine the combination of the following variables giving the most efficient and rapid deoxygenating rate:

Variable	Level
Glucose concentration (buffered at pH 5.1 - optimum pH recommended by manufacturers of enzyme concentrate)	30 g/l**, 300 g/l, 750 g/l
Enzyme concentration	15, 30, 90, 125 units/ml. of buffered glucose solution
Absorbant	synthetic sponge, molecular sieves, diatomaceous earth purified SiO_2, T-1 absorbant (ground cereal)

*"A glucose oxidase unit is defined as that quantity of enzyme which will cause the uptake of 10 mm^3 of oxygen per minute in a Warburg manometer at 30°C in the presence of excess oxygen with a substrate containing 3.3% of glucose monohydrate and phosphate buffer, pH 5.9."[19]

**30 grams per liter, - - - - - etc.

Strips of synthetic sponge 3/4" x 3/4" x 2" were placed in 250 cc French square bottles. To two solutions containing 5 ml each of glucose (30 g/l and 300 g/l, respectively), 1 ml and 0.5 ml enzyme concentrate was added. The sponges were saturated with this solution, manometers affixed and the bottles sealed. Manometer readings were made at recorded intervals. The above experiment was repeated using the additional absorbant materials and various glucose/enzyme concentrations described above. Figure 5 shows the manometric apparatus used in determining the deoxygenating efficiency

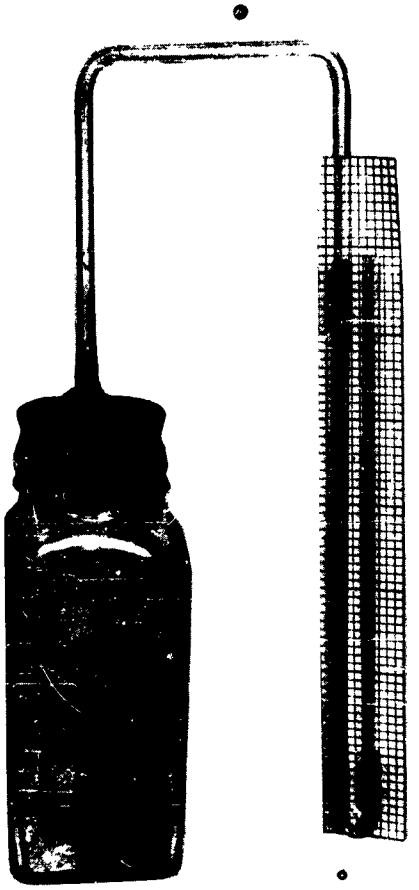


FIGURE 5 Neg.No. 3351

MANOMETRIC APPARATUS UTILIZED

and oxygen uptake when several levels of glucose and enzyme concentration and various absorbants were investigated. Figure 6a shows the effect of varying the concentration of both the glucose and the enzyme. From this initial test it was apparent that slightly higher enzyme concentration and higher concentration of glucose should provide sufficiently rapid deoxygenation. Figure 6b shows the effects of varying the glucose and enzyme concentrations as well as the absorbant for the deoxygenating media. It is readily apparent from this figure that of the several materials and concentrations tested, none appeared to be as efficient as the synthetic sponge material using a 750 gram per liter concentration of glucose and 3 mls of the enzyme concentrate. The enzyme solution was pipetted onto the surface of the sponge, but in the case of the silica gel, diatomaceous earth, and

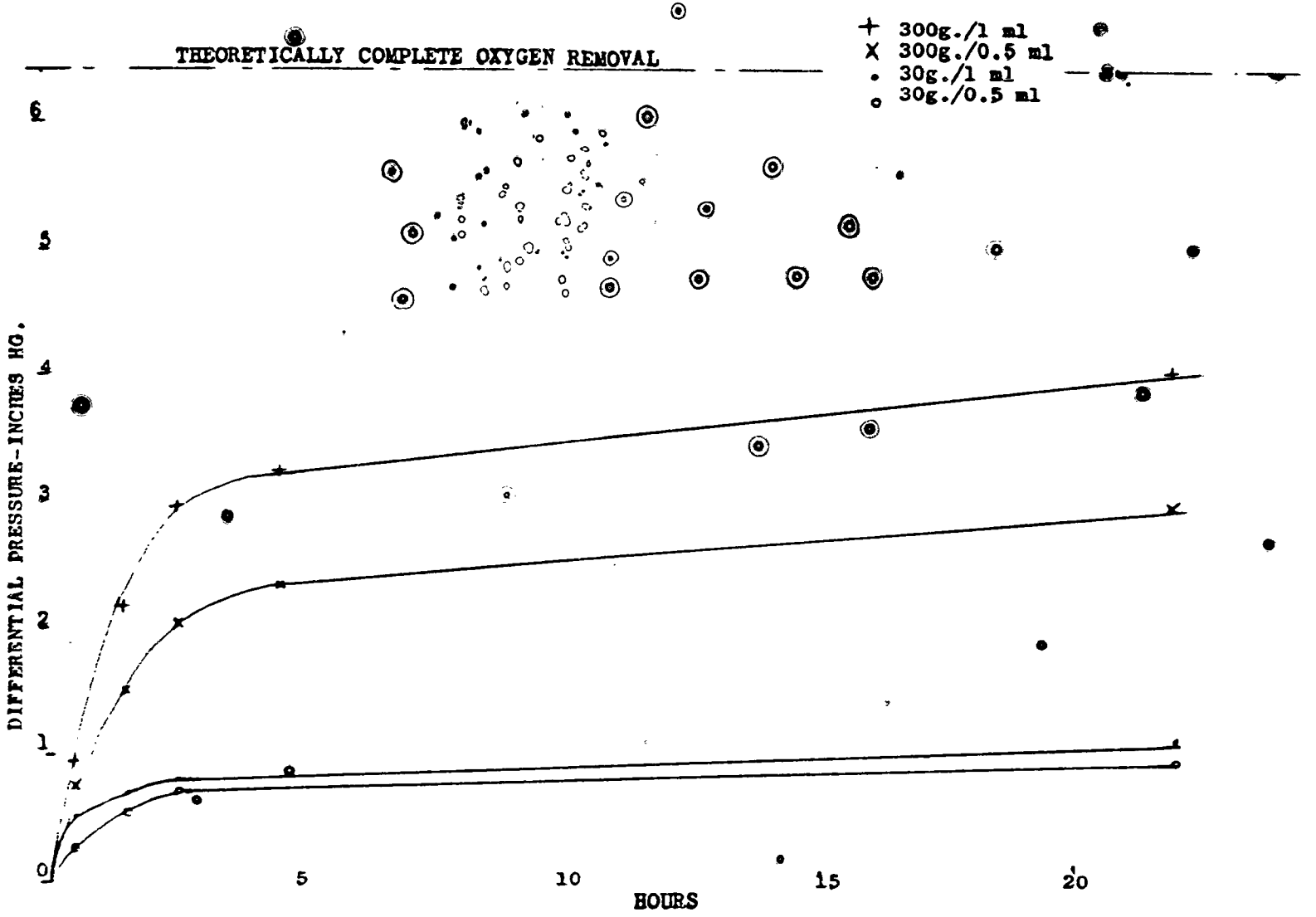


FIGURE 6a

OXYGEN REMOVAL-VARYING LIQUID ENZYME/GLUCOSE CONCENTRATION ON SYNTHETIC SPONGE

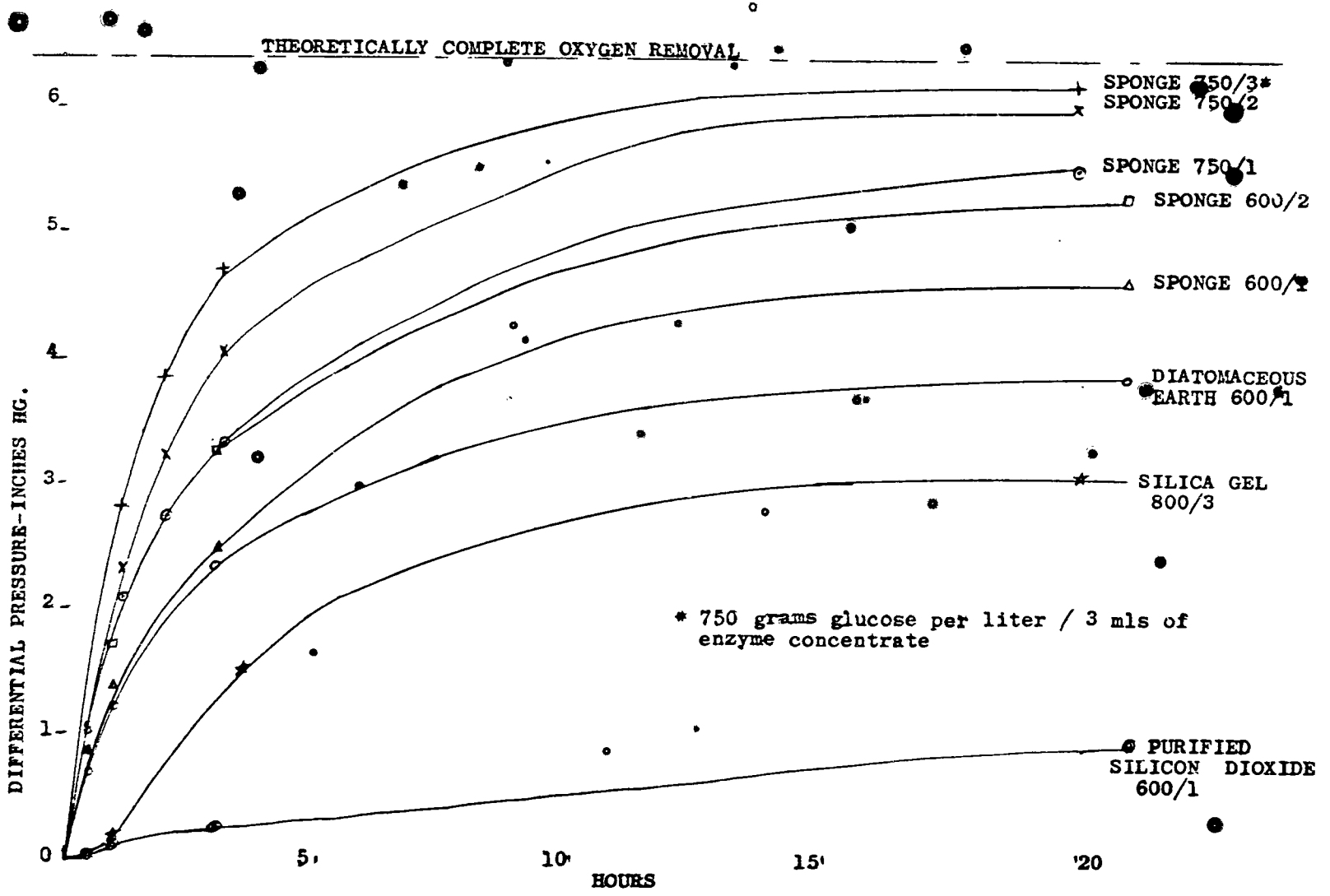


FIGURE 6b

OXYGEN REMOVAL-LIQUID ENZYME/GLUCOSE CONCENTRATION VS. ADSORBENT MATERIALS

purified SiO_2, the enzyme mixture was stirred into these absorbants in an effort to deposit the enzyme system on a large surface area. The purified SiO_2 absorbed very poorly as the graph shows. Using the sponge as an absorbant, resulted in a greater deoxygenating rate and capacity.

A comparison was made between synthetic sponge and molecular sieves (alkali metal alumino-silicates similar to many natural clays and feldspars) as substrates for the enzyme system. Two mls of a 1:5 enzyme concentrate/saturated glucose solution were placed in each of two test tubes. Approximately 3 grams of molecular sieves were added to the tubes resulting in an exothermic reaction. After gas evolution had ceased, a slight excess of the sieve was added and the mixture shaken vigorously to adsorb all surface liquid. The loaded sieves were then transferred to 50 cc. glass containers and manometers affixed. Two mls of the 1:5 solution was also added to two sponge pieces 1 x 1 x 3 cm. Each sponge was placed in a 50 cc container as described above. A fifth container, used as a control, contained two mls of the 1:5 solution.

A comparison of the 1:5 enzyme concentrate/saturated glucose solution on the molecular sieves and on the synthetic sponge is shown in Figure 7. The deoxygenating solution itself was used as the control. As the figure shows, the deoxygenating rate is most rapid using the sieves as the absorbant, approximately 5.4 inches of Hg. in one hour compared to approximately 4.7 inches Hg. using sponge as the absorbant. The control deoxygenated much slower due to its small surface area as compared to the sponge and sieves. After 18-20 hours the difference in rate of oxygen uptake between the sieves and sponge was relatively minor. The advantages of using the sieves were the rapid initial oxygen uptake and the added benefit of the sieves being dry.

Several materials including paper/plastic laminates and unsupported plastic films were investigated as containers for the deoxygenating system.

One and one-half inch by 2-1/2 inch prototype deoxygenating packets were fabricated from 1/2 mil low density polyethylene, polyester film, polyvinylidene chloride, and metallized polyester. Each packet contained 4 mls of a 1:5 enzyme concentrate/saturated glucose solution loaded on approximately 7 grams of molecular sieves. For comparison purposes, Figure 8 shows the deoxygenating efficiency of the prototype packet versus the same amount of deoxygenating solution deposited on synthetic sponge (3/4" square by 2") and sealed in low density polyethylene packets. The packets

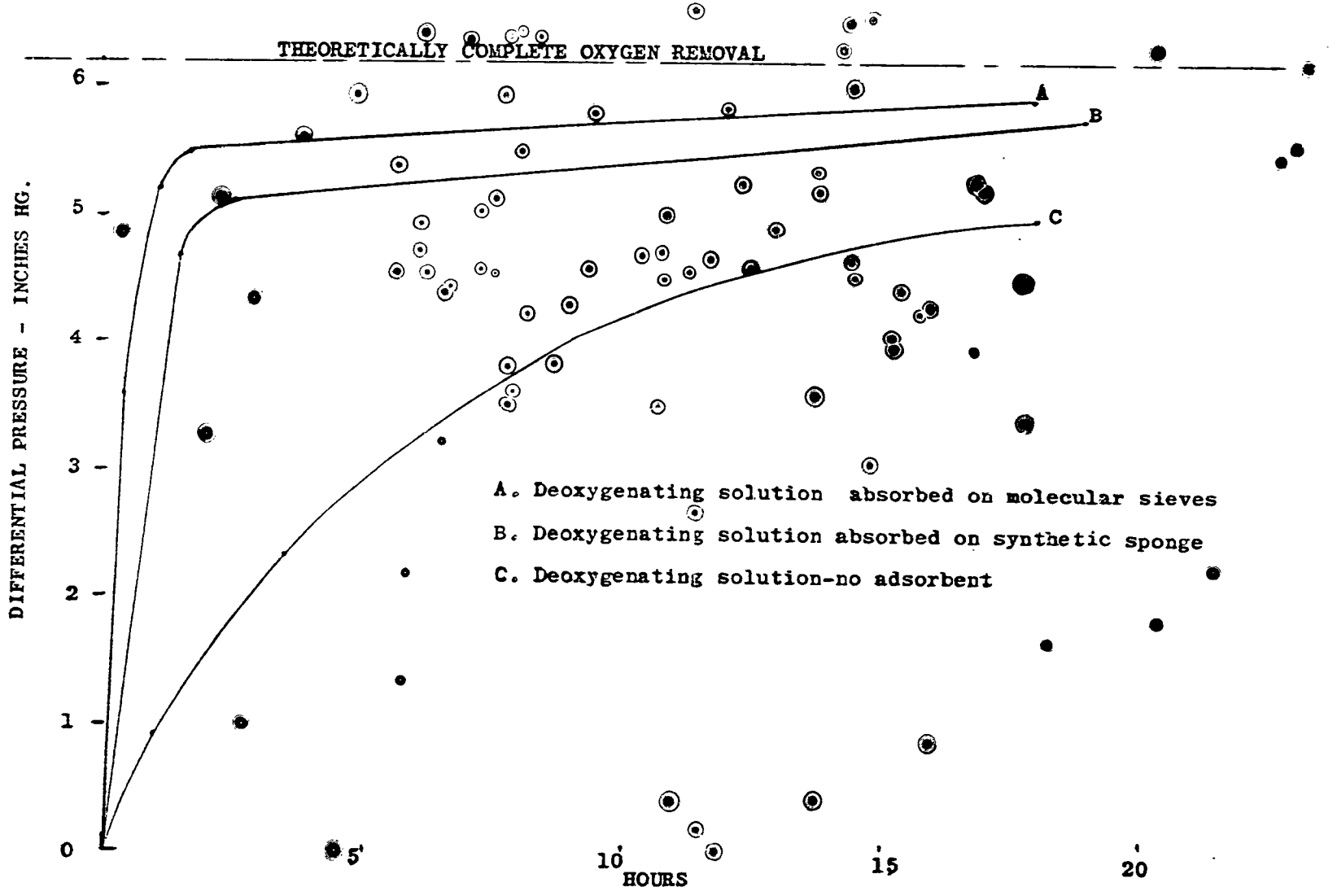


FIGURE 7

OXYGEN REMOVAL-MOLECULAR SIEVES VS. SYNTHETIC SPONGE

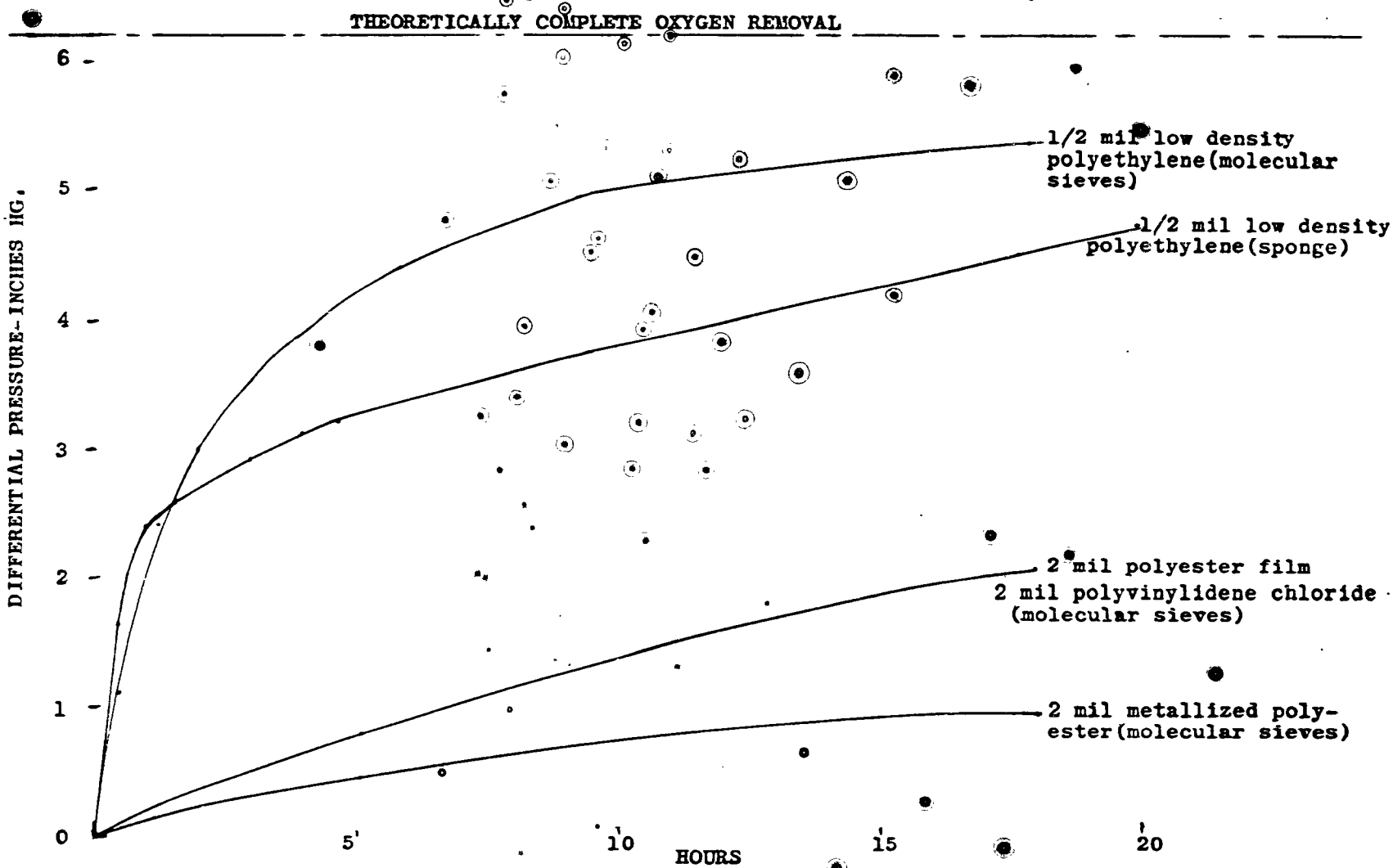


FIGURE 8

OXYGEN REMOVAL-UTILIZING VARIOUS PACKET FORMING PLASTIC FILMS

were placed in 100 cc. glass containers and manometers affixed as described previously. The deoxygenating rate was determined from the manometer readings taken at recorded intervals. The above figure also shows the efficiency of the deoxygenating ingredients loaded on sieves and contained in mylar, saran and aluminized polyester.

Of the materials tested, i.e., mylar, saran, polyvinyl chloride, aluminized polyester, kraft/polyethylene laminate, and low density polyethylene, only the latter was considered an effective packet material. For rapid uptake of oxygen, it was necessary to use a material which had a high gas permeability rate, and at the same time was liquid light. Low density polyethylene had the best combination of high oxygen permeability and low moisture vapor transmission of the materials tested.

While the prototype packet as described above proved the feasibility of a simple deoxygenating device, several flaws in any practical application of such a packet were obvious, namely; the packet's fabrication was slow and inefficient (due to heat build-up and gassing), also the packets had to be used immediately after being made up and as such had no shelf life.

In an effort to improve the prototype packet an investigation was initiated into increasing the rate of reaction and shelf life of the deoxygenating packet.

As shown in Figure 8, approximately 18 hours were required for the prototype packet to deoxygenate to 5.2 in. Hg. In order to improve the rate of oxygen absorption, a surfactant, A, was added to the saturated glucose solution prior to absorption on the molecular sieves. A small amount of the surfactant was placed in 25 mls of saturated glucose solution and stirred for ten minutes. To 3.3 mls of this solution, 0.67 mls of enzyme concentrate was added and deposited on 3 grams of molecular sieves in a test tube. Again, as described previously, an exothermic reaction occurred. After gassing had ceased, the stoppered tube was shaken vigorously to adsorb all surface liquid. When cool, the loaded sieves were transferred to a 100 cc. container, a manometer affixed and sealed. As indicated in Figure 9, complete oxygen removal was not obtained, however, a vast increase in the deoxygenating rate was affected. The data on the experiment described above were based on the use of a particular uncoated molecular sieve. Further work utilizing the surfactant was performed using an "improved type" of molecular sieves from the same manufacturer. The "improved" sieves were clay coated for crush resistance which inhibited

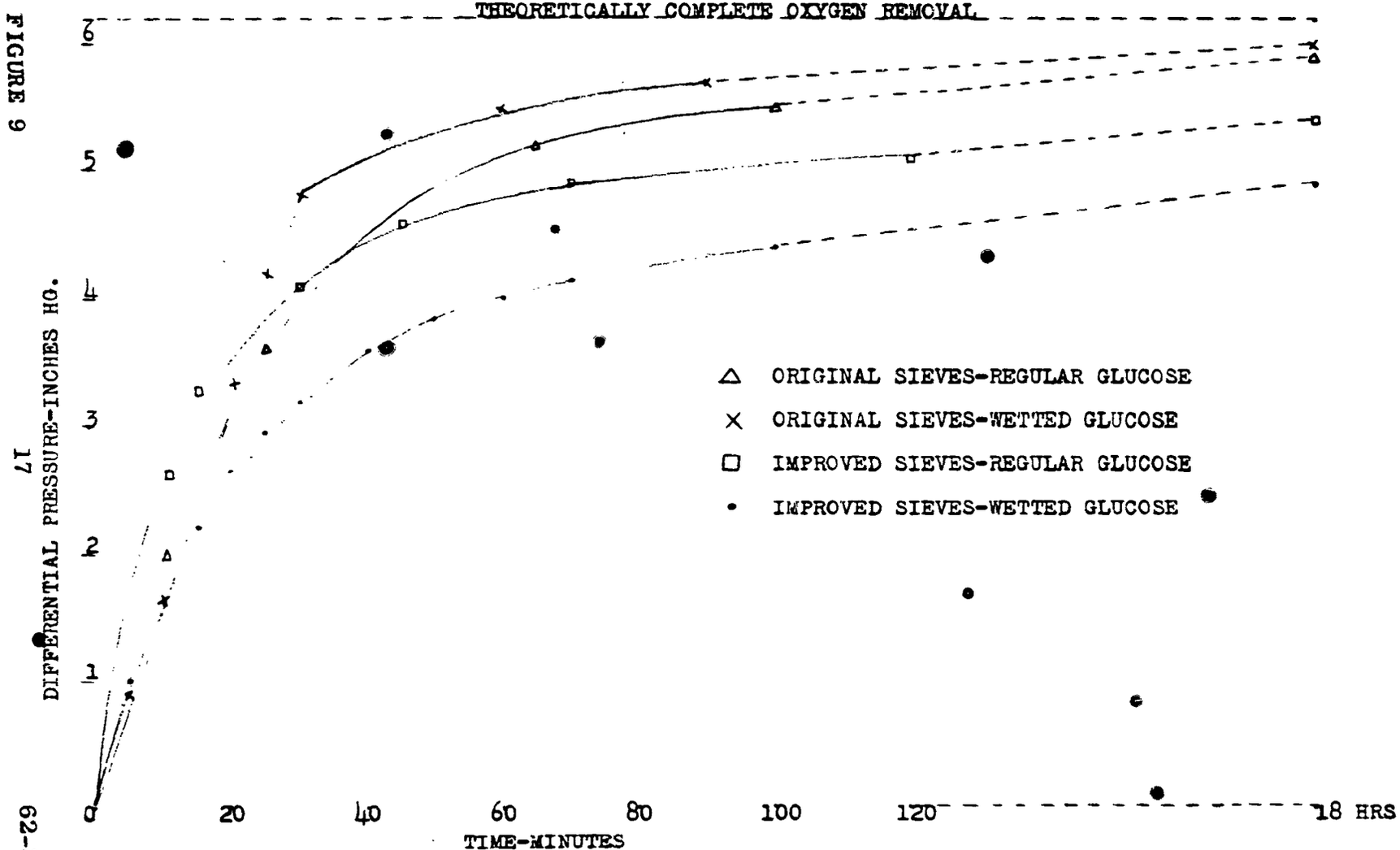


FIGURE 9

COMPARING THE EFFECTS OF THE ADDITION OF A SURFACTANT
TO BOTH ORIGINAL AND "IMPROVED" MOLECULAR SIEVES

62-3441

absorption of the deoxygenating solution (see Figure 9). The above figure also shows that addition of the same surfactant on the new sieve did not result in improved deoxygenating rate or capacity.

In view of the fact that the above mentioned surfactant did not improve the rate of oxygen absorption or capacity of the systems, two other surfactants were evaluated utilizing the "improved" molecular sieve. The originally received molecular sieves were not evaluated in this experiment since not enough material was available.

Figure 10 shows a comparison of the three surfactants used in this experiment. The latter two surfactants were applied to the deoxygenating system in the same manner as the first. Surfactant B appeared to be somewhat faster than A, while C offered no increase in rate or capacity to the deoxygenating system. No deoxygenating packets were made up from the improved sieves and the various surfactants.

Since the original substrate for the deoxygenating liquid was no longer available and the "improved" product was not satisfactory, several other materials were evaluated as substrate for the deoxygenating solution.

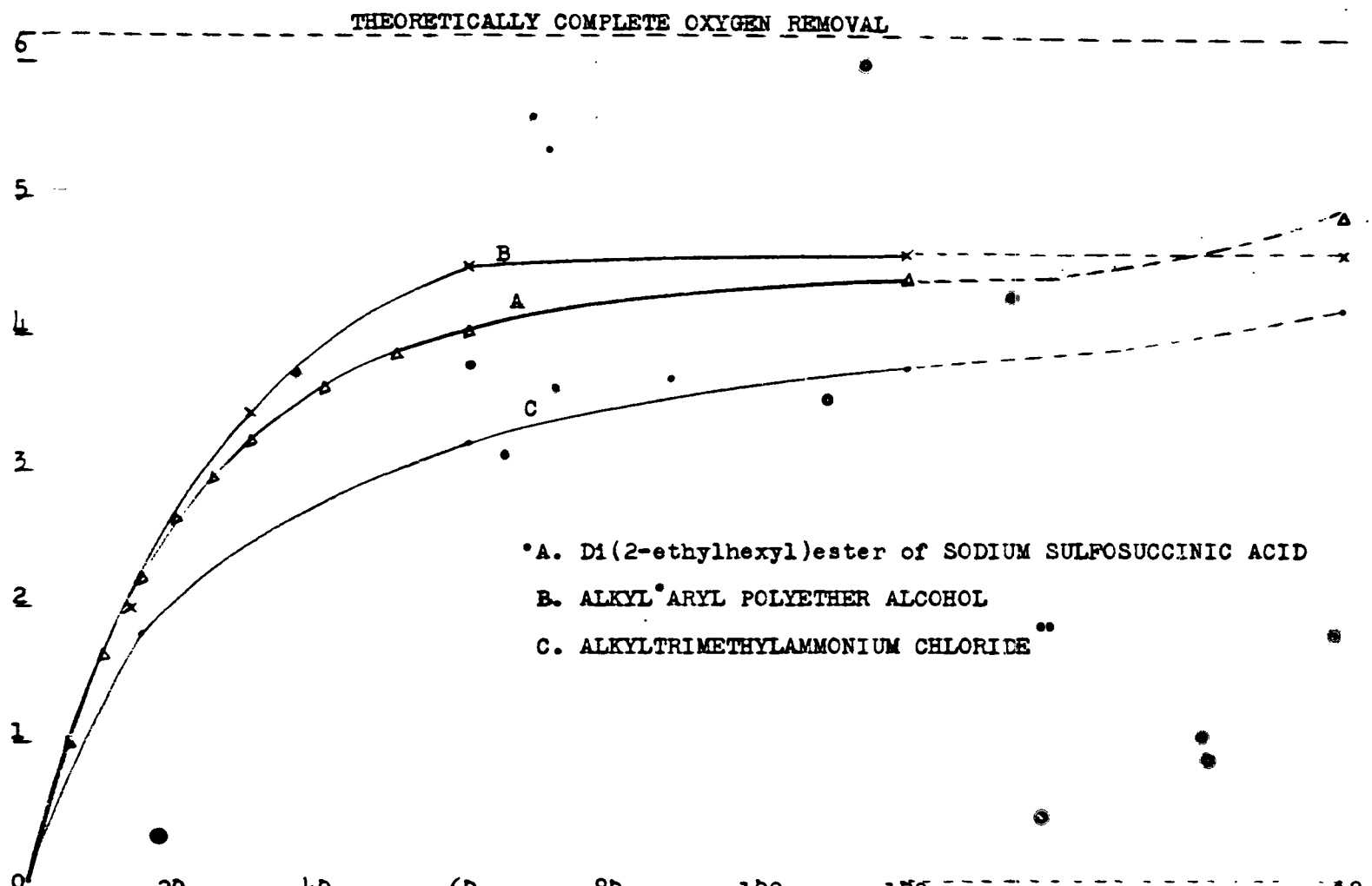
Substrates

The molecular sieves as first used were obtained in pellet form (approximately 1/16" dia., 3/16" long). This material was also available in 1/8" pellets as well as in powder form. Figure 11 shows the deoxygenating rate and capacity of the molecular sieves as 1/16" and 1/8" pellets and in powder form. The deoxygenating solution was added to the 1/8" pellets in a test tube as described previously. Enough powdered sieve was added to 3.4 ml of the buffered saturated glucose solution to make the mixture dry and free flowing. The glucose loaded powdered sieve was placed in the 110 cc. container, 0.67 ml of enzyme concentrate added, manometer affixed and sealed.

It is readily apparent from the figure that the 1/8" pellets and the powdered sieve do not compare in any way to the 1/16" pellets. Aside from their indicated poor deoxygenating rate and capacity, these forms of molecular sieves were extremely difficult to wet with the deoxygenating solution.

Figure 11 also shows the deoxygenating rate and capacity of the enzyme system when deposited on a second synthetic zeolite (#2) material similar to molecular sieves.

FIGURE 10



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OXYGEN REMOVAL UTILIZING SEVERAL SURFACTANTS AND IMPROVED SIEVES

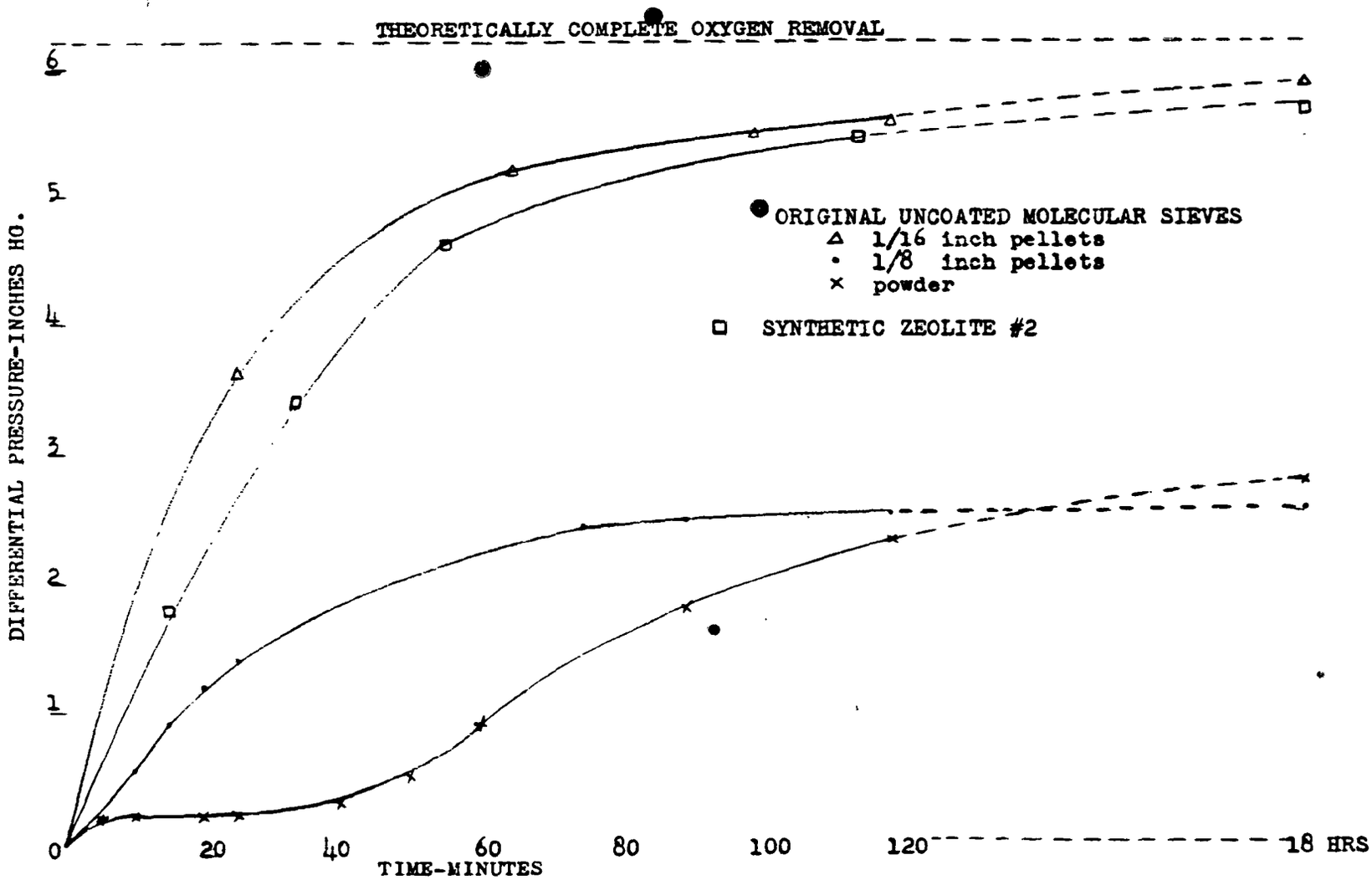

FIGURE 11

OXYGEN REMOVAL COMPARING THREE FORMS OF UNCOATED MOLECULAR SIEVES AND SYNTHETIC ZEOLITE #2

The second zeolite substrate was obtained in an aggregate-bead form with a surface area greater than that of the molecular sieves. It was expected that this second synthetic zeolite as a substrate for the deoxygenating solution would provide for a faster rate of oxygen absorption with capacity equivalent to molecular sieves. However, the figure indicates the molecular sieves to be the better of the two, both in regard to rate of oxygen absorption and capacity. The second zeolite material was not utilized in a deoxygenating packet since it presented the same draw backs as the molecular sieves.

Additional work utilizing molecular sieve type substrates did not result in the necessary oxygen uptake. A further deterrent to the use of molecular sieves was their high cost.

Evaluation of a commercial activated alumina proved to be rewarding, both efficiently and economically. The alumina obtained was a crystalline dehydrating agent in ball form (1/4" by 8 mesh). This desiccant appeared to have several salient features necessary for an effective substrate, i.e., chemically inert, extremely adsorbant, large surface area, and inexpensive.

Initial tests using alumina balls as a substrate proved to be unsuccessful. The deoxygenating liquid (.67 ml enzyme concentrate/3.4 mls saturated-buffered glucose solution) was placed in a test tube, approximately 16 grams of alumina added and the test tube stoppered. The liquid did not adsorb on the alumina as expected and consequently when added to the manometer set-up was still wet. Of importance here, however, was the fact that very little heat build-up and gassing occurred.

The above experiment was repeated except that the deoxygenating solution was poured on the alumina while in the manometer set-up. After a slight initial pressure build-up was released the manometers were sealed and containers set aside to deoxygenate. Figure 12 shows the deoxygenating rate and capacity of the system when utilizing activated alumina as the substrate. The figure shows that while the deoxygenating rate using the alumina was not as rapid as when molecular sieves were used, the ultimate capacity was far greater.

No explanation is given for the high reading recorded after 18 hours deoxygenation. All values recorded were those corrected against a control manometer. A reading of 6.2 inches Hg. would have indicated complete oxygen removal.

Figure 13 schematically shows a fabricated packet utilizing activated alumina as the substrate for the deoxygenating

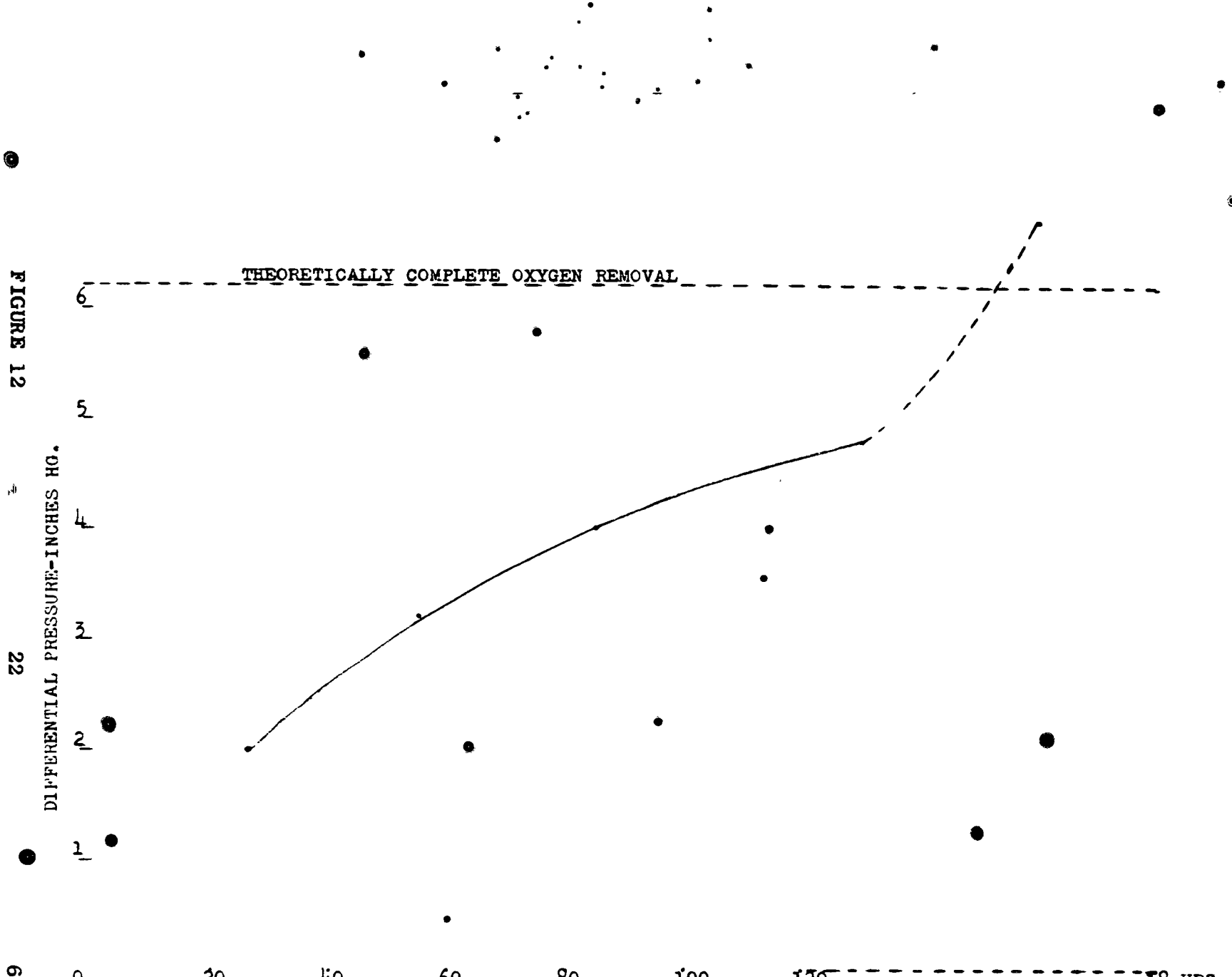

FIGURE 12

RATE OF OXYGEN ABSORPTION AND CAPACITY OF THE DEOXYGENATING SYSTEM WITH ACTIVATED ALUMINA AS THE SUBSTRATE

solution. The packet is of 1/2 mil low density polyethylene, 2 inches by 2-1/2 inches. The glucose loaded substrate is placed in the packet and a smaller sealed packet containing 0.7 mls of enzyme concentrate added. The large packet is then heat sealed. For deoxygenating purposes, the small inner bag of enzyme is ruptured by squeezing, thus allowing the enzyme and glucose to react.

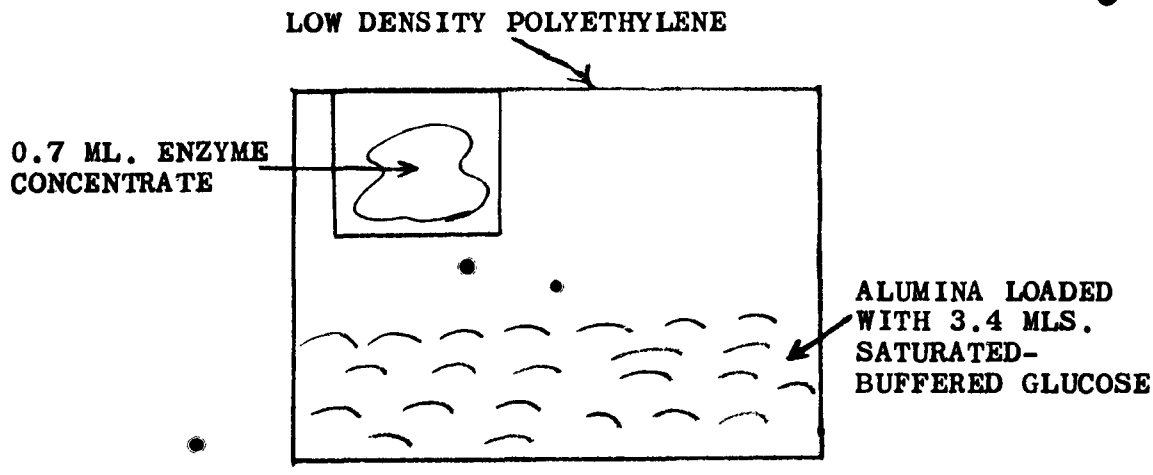

FIGURE 13

SCHEMATIC DIAGRAM OF THE "IMPROVED" DEOXYGENATING PACKET

In the initial packet, the alumina was loaded while in a test tube with 3.4 mls of glucose. The deoxygenating rate of such a packet was considerably slower than expected. In an effort to improve the deoxygenating rate, alumina was immersed in the glucose solution for approximately 15 minutes. The liquid was decanted off, and the alumina allowed to air dry. Packets were made up using the alumina loaded in each way. Figure 14 compares the rate and capacity of packets using "loaded" and saturated alumina. While the capacity of both types of packets is nearly the same, packets containing glucose saturated alumina effect a more rapid deoxygenating rate.

Figure 15 shows the improvement in deoxygenating rate and capacity of the "improved" packet containing glucose saturated alumina over the prototype packet. This figure

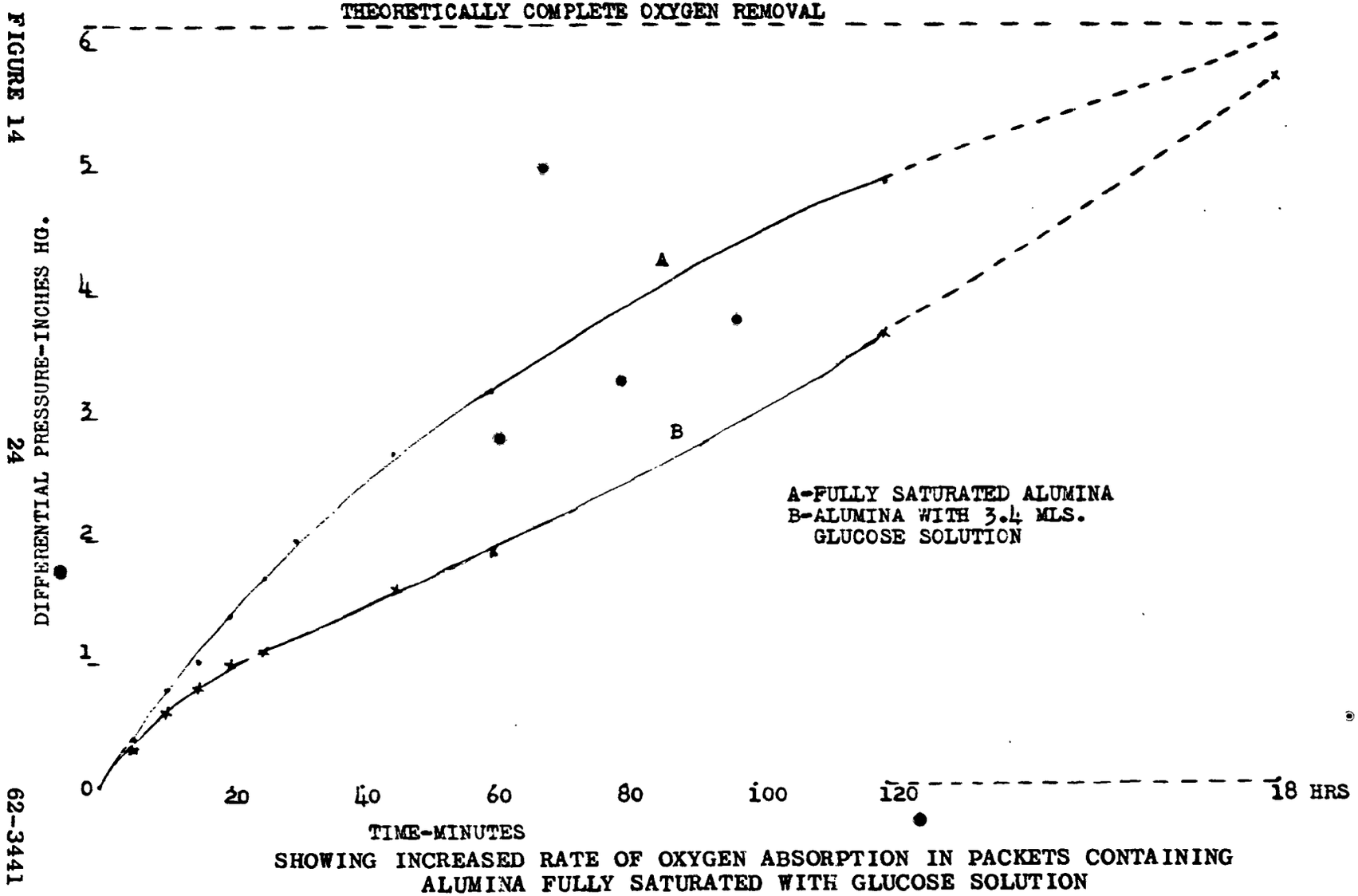

FIGURE 14

SHOWING INCREASED RATE OF OXYGEN ABSORPTION IN PACKETS CONTAINING ALUMINA FULLY SATURATED WITH GLUCOSE SOLUTION

62-3441

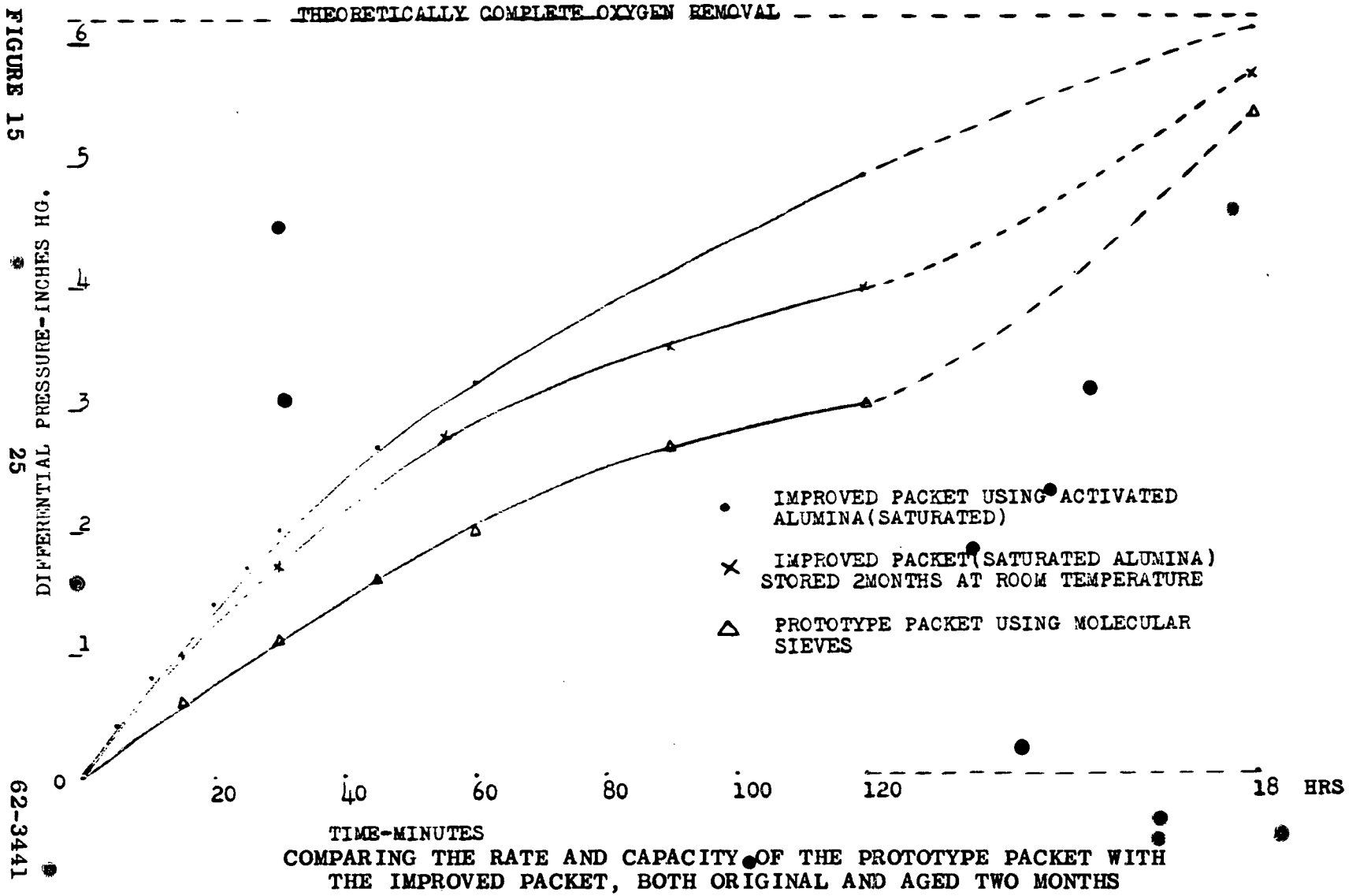

FIGURE 15

COMPARING THE RATE AND CAPACITY OF THE PROTOTYPE PACKET WITH THE IMPROVED PACKET, BOTH ORIGINAL AND AGED TWO MONTHS

also indicates that the packet after 2 months storage at room temperature loses approximately 5% of its deoxygenating capacity. Storage tests were not continued beyond this period.

It can readily be seen that the alumina-packet is a definite improvement over the prototype seive-packet. The former is relatively easy to fabricate and has a definite shelf life while the latter must be used immediately and is much more difficult to make up.

The improved packet described above is readily adaptable to all phases of rigid container packaging. As has been previously stated, the removal of oxygen from a system would allow more variation in the packaging of previously non-compatible metals as well as items which cannot be contaminated by any type of preservative.

This enzyme deoxygenating system is also thought to be adaptable to flexible packaging (plastic films). However, use of a deoxygenating system for corrosion prevention with flexible packaging films creates the requirement that the system be in the form of a coating, since some air will enter the package in the course of time and this air must be deactivated by the deoxygenating medium prior to reaching the protected item if corrosion is to be prevented. Available information[20] supports the contention that microencapsulation of the deoxygenating system into small capsules (125 microns in diam.) coated on the interior surface of plactic barrier materials would result in deoxygenation of the interior and would prevent the entrance of oxygen into the protective atmosphere within the pack.

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$$2\ C_6H_{12}O_6 + 2\ O_2 + 2\ H_2O \xrightarrow[\text{oxidase}]{\text{glucose}} 2\ C_6H_{12}O_7 + 2\ H_2O_2$$

glucose → gluconic acid

$$2\ H_2O_2 \xrightarrow{\text{catalase}} 2\ H_2O + O_2$$

Net reaction:

$$2\ C_6H_{12}O_6 + O_2 \xrightarrow[\text{system}]{\text{enzyme}} 2\ C_6H_{12}O_7$$

Three surfactants were evaluated in a saturated buffered glucose solution with a view toward increasing its absorption on the molecular sieves, and the capacity and rate of oxygen absorption of the deoxygenating medium. Surfactant A proved to be the most effective wetting agent used; increasing both the capacity and rate of the original mixture.

Four forms of molecular sieves (1/16", 1/8", powdered and "improved") a second synthetic zeolite and activated alumina were investigated as substrate material for the deoxygenating liquid. Of the several materials tested, activated alumina provided the best balance of substrate properties, inert, highly absorbant, and inexpensive. An improved deoxygenating packet utilizing activated alumina as the substrate material for the deoxygenating liquid is described.

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